NEW YORK STATE COLLEGE OF AGRICULTURE
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LABORATORY OF BACTERIOLOGY

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Dear Josh:

I have delayed writing until I have had a chance to play with the bug a bit. The cultures arrived OK and I think I have isolated the proper organism. I have had four sessions of single celling so far starting with two or more original cells from a + EMS colony each time (EMS using lactose and succinate). All the original cells have been heterozygotes. No interesting results were obtained until the last attempt and I shall describe them later.

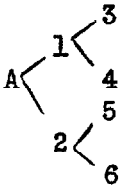
The technique appearing best at the moment is: get original cells for a + EMS colony. After the separation of the cells they are allowed to form microcolonies which are then picked up and suspended in 1/2 ml. of a synthetic medium lacking a carbon source. A drop or two, depending on the size of the microcolony is spread over an EMS and an EMB plate with glass rods immediately. Then an equal amount of double strength broth is added and the tubes incubated until turbidity. This early plating minimizes the chance for misclassifying a cell by not plating until after the broth culture becomes turbid allowing more opportunity for segregation. For example, on the first set where I did not plate immediately I got a culture appearing to give all +$^{\sigma}_{1}$EMB. A few + colonies on EMS, however, gave mosaics when subsequently plated on EMB.

I have been classifying the cultures as follows:
If even one mosaic colony is obtained on EMB I consider the cell from which the culture was obtained to have been a heterozygote.

If the culture gave no mosaic but some + and some - it would also be classified a heterozygote. Similarly, if one + EMS colony is obtained which segregates I call the cell a heterozygote. Classifying a cell as a segregant to + or - seems less exact. However, if plated early as above and if a 100 or 1000 colonies were observed with no mosaics but all + or - as the case might be, it would seem safe to consider the cell as a segregant. Have you any comments or advice on this?

My system of numbering cultures has been as follows:
I carry the date and a letter, A, B, etc., to designate the original cell. Progeny of the original cell are designated with the same date and letter plus a number, as follows; the progeny of a cell always being twice the

parent cell + 1 and + 2:



Thus from culture numbers you can readily reconstruct the pedigree.

I have had some difficulty with cells not growing, the series presented later being by far the ~~most~~ worst in this respect. Occasionally cells grow but don't divide, forming long filaments which may or may not ultimately produce a microcolony. Sometimes the filament cuts off a cell from one or both ends which might, but usually doesn't, grow. I have had cells split up into three cells, apparently simultaneously, all of which messes up the nice system of designating cell relationships. I tried to note all such irregularities in behavior.

I don't understand all I know about the culture and would appreciate any information you can give me about classifying the cultures. So far, I have never plated a + EMS colony without it producing largely mosaics with a few + and a few - colonies. Is this necessarily so, or is it a happy chance due to the rather small number of such colonies I have plated so far? If the cell is heterozygous for several nutritional factors only a rare crossover segregant would be able to grow on EMS. What nutritional factors have you determined ~~would~~ to be heterozygous in H-72? I have not done a great deal of plating with various types of colonies but + always seems to give + while - plated gives - at first and later secondary + growth develops in some colonies. Hence, it appears that reverse mutation occurs. Have you studied this?

Have you any advice on the simplest method of checking the nutritional requirements of the cultures? I feel it would be desirable to check them here at least partially and on younger cultures. Further, I would like to do enough of it to master the techniques since I have never worked with biochemical mutants.

Now to the interesting case. In the pedigree of cell A of February 26, 1949:

Terminal
~~Technical~~ cells which did not grow: 4, 31, 32, 33, 34, 17, 37, 52, 53, 13, 14.

Terminal
~~Technical~~ cells classed as heterozygotes, giving mosaics on EMB and some + on EMS: 77, 78, 219, 220, 221, 222.

~~Technical cells classed as ?, giving mosaics on EMB but no colonies on EMS:~~ 51, 53. (on replating 51, 53)

Terminal
~~Technical~~ cells classed as - segregants: 47, 48, 99, 100, 101, 102.

Cell 51 did not grow for a considerable period, finally did divide to form a few cells then apparently stopped growing. I picked the colony anyway, plated nearly the whole 1/2 ml. between the EMS and EMB plates. Got no colonies on EMS, six mosaics and one negative on EMB. The tube itself showed no growth when incubated. The culture labeled A-51 is a transfer of one of the mosaic colonies from the EMB plate. No. 53, however, had upwards of 200 mosaic colonies on the EMB plates but none on the EMS plates. I am checking this result. Should there not be equal numbers of mosaic colonies on EMB and + colonies on EMS if the plating were quantitatively equal?

The cultures classed as - segregants grew normally in the moist chamber and the EMB plates were crowded, say 2000 small colonies, with no + colonies. The EMS plates gave nothing. All six cultures gave identical results with from 20 to 40 per plate of larger more opaque colonies appearing - at 48 hours but showing + centers at 72 hours. Reverse mutation or what? At any rate, I am shipping the whole A series to you which brings up the question of how best to send cultures. Offhand, it seems desirable to get them to you with the minimum amount of growth and postage so I am sending them in small tubes. I am sending small slants and also a tube containing equal parts of the original broth culture to which an equal amount of 2% agar was added. Would it be better to seal off the tubes and send the original broth cultures? Until I hear from you I shall continue playing with the culture and do more single cell isolations as time permits. I hope I am not too incorrect in the way I have been classifying the cultures. I will look forward to getting more information about the culture in return mail.

One of the grad students here is interested in obtaining a biotin deficient strain of coli. Do you have any such available, or know where he might obtain one?

Very truly yours,

M. R. Zelle

MRZ:jc

P.S. Have you estimated the frequency of the "segregation" from the proportion of mosaic to non-mosaic colonies? If so, about what. Also, have you a convenient method of counting cells?

Apologies for the sloppy letter, our steno is not the best & I thought of something else every 5 minutes.

How did H-72 originate?

Why the ruminate in the EMS along with lactose?

Platings from the entire colonies grew mostly + with some - colonies. Haven't plated from the edge. No growth on EMS.